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Characterization of lignin-derived products from Japanese cedar as treated by semi-flow hot-compressed water

Masatsugu Takada • Shiro Saka

Affiliation

Department of Socio-Environmental Energy Science, Graduate School of Energy Science, Kyoto University, Yoshida-honmachi, Sakyo-ku, Kyoto 606-8501, Japan

Tel. +81-75-753-4738; Fax: +81-75-753-4738

e-mail: saka@energy.kyoto-u.ac.jp

Abstract

In order to elucidate the decomposition behavior of lignin from Japanese cedar (*Cryptomeria japonica*) as treated by two-step semi-flow hot-compressed water (1st stage: 230 °C/10 MPa/15 min, 2nd stage: 270 °C/10 MPa/15 min), water-soluble portion,

precipitate and water-insoluble residue obtained by hot-compressed water treatment were separated and characterized. Consequently, the water-soluble portion was found to contain lignin-derived monomeric compounds such as coniferyl alcohol and coniferyl aldehyde and β -1, β -5 and 5-5' linked dimeric compounds. These lignin-derived compounds maintained methoxyl and phenolic hydroxyl groups in aromatic rings. The water-soluble portion also contained lignin-derived oligomeric compounds up to heptamers. In contrast, the precipitate was found out to consist of higher molecular weight lignin with high ether type linkages. The water-insoluble residue, however, consisted mostly of lignin with high condensed type linkages. Based on these lines of evidence, condensed type lignin must be resistant to hot-compressed water, remained as water-insoluble residue after two-step treatment. Such information provides a clue as to efficient utilization of lignin-derived products.

Keywords Japanese cedar, Hot-compressed water, Lignin-derived products, condensed type lignin

Introduction

The wood cell wall consists mainly of cellulose, hemicelluloses and lignin, and these compounds are composed intricately in an artful manner to enforce the wood cell wall, having a resistance to water and most organic solvents. Among the natural polymers, lignin is the second most abundant macromolecule with 24-33 % in softwoods and 19-28 % in hardwoods [1]. Since lignin may become a resource of energy and valuable aromatic chemicals due to its abundance, various degradation methods have been studied for its utilization. Among them, hydrothermal treatments such as steam explosion, subcritical and supercritical water and hot-compressed water are catalyst-free and environmentally benign. An increase in temperature of water at a high pressure results in a decrease in its dielectric constant and an increase in its ionic product [2,3]. Moreover, decreased dielectric constant of water enhances solubility of some hydrophobic compounds into subcritical and supercritical water.

Tanahashi et al. examined the degradation mechanism of lignin by steam explosion (230 °C/2.9 MPa/16 min) with dehydrogenated polymer of coniferyl alcohol and β -O-4 linkage model compounds [4]. Ehara et al. [5] and Takada et al. [6] treated Japanese beech (*Fagus crenata*) and Japanese cedar (*Cryptomeria japonica*) with supercritical water (380-400 °C/100-115 MPa/5-8 sec). Consequently obtained lignin-derived

compounds were fractionated as methanol-soluble portion but water-insoluble oily products were composed of monomeric aromatic and condensed type dimeric compounds. Yokoyama et al. treated organosolv lignin with supercritical water (350-420 °C/10-40 MPa/60 min), and the resultant yields of oil and char were dependant on the treatment condition [7]. Saisu et al. [8] and Okuda et al. [9] also treated organosolv lignin with supercritical water (400 °C/pressure not given/10-64 min) and obtained reactive compounds such as formaldehyde and lignin-derived monomeric compounds such as guaiacol, catechol and phenol were obtained due to hydrolysis and the subsequent dealkylation. They explained that low molecular weight compounds were reactive such that they reacted with residual lignin to become high molecular weight residue.

Phenol and aromatic nuclear are known to be stable to supercritical water treatment through some experiments with various monomeric lignin model compounds [10-15]. The resistance of lignin to supercritical water differs, dependent on the types of its linkages, and ether linkages in lignin are readily cleaved by protic solvent such as supercritical water and methanol, while condensed type of linkages and aromatic ring are difficult to be cleaved [16,17].

Recently, hot-compressed water, a milder condition for hydrothermal reaction with high ionic products, has gained considerable attention as a promising decomposition

medium for lignocelluloses [3], and the hot-compressed water treatment system could be simply categorized into batch, flow, and semi-flow (percolation) types. Since the decomposition reaction becomes dominant in batch and flow type treatments, some products are excessively decomposed, whereas semi-flow type treatment can prevent products from excessive decomposition due to the short residence time [18,19]. Lignin also can be depolymerized and decomposed by semi-flow hot-compressed water [20].

Lu et al. treated Japanese beech (*Fagus crenata*) with two-step semi-flow hot-compressed water (1st stage: 230 °C/10 MPa/15 min, 2nd stage: 270 °C/10 MPa/15 min) and the hemicelluloses and cellulose were selectively decomposed [21]. They also elucidated that lignin was decomposed in both stages and the lignin-derived monomeric products such as coniferyl alcohol and sinapyl alcohol were obtained in water-soluble portion [21]. Yamauchi et al. showed that hot-compressed water can cleave only ether linkages of lignin through the structural analysis of lignin-derived products [22]. Additionally, these lignin-derived products could be converted into acetic acid and phenolic compounds via microorganism, thus, these are expected to become valuable chemicals [23].

Phaiboonsilpa et al. treated Japanese cedar (*Cryptomeria japonica*) and obtained lignin-derived products with hot-compressed water [24]. However, detailed analysis of the lignin-derived products obtained from Japanese cedar has not yet been conducted.

Yamauchi et al. have studied the decomposition behavior of lignin in Japanese beech as described above [22], while lignin content and its structure in Japanese cedar are different from Japanese beech, and their decomposition behaviors of lignin should also differ. Thus, the aim of this study is to analyze the lignin-derived products in order to elucidate the decomposition behavior of lignin from Japanese cedar as treated by hot-compressed water.

Materials and methods

Wood sample and chemicals

Wood flour of Japanese cedar (*Cryptomeria japonica*) passing through an 18-mesh screen was extracted with acetone using Soxhlet apparatus, and dried at 105 °C for 24 h before experiment. All chemicals used in this study were of reagent grade without purification.

Two-step semi-flow hot-compressed water treatment

Approximately 0.5 g of oven-dried extractive-free wood flour was placed in the reaction vessel for which the two-step semi-flow hot-compressed water treatment was performed as described in a previous paper [24]. Two-step treatment conditions were 230 °C/10 MPa/15 min for the 1st stage and 270 °C/10 MPa/15 min for the 2nd stage. After treatment, hot-compressed water-insoluble residue left over in the reaction vessel was separated from hot-compressed water-soluble portion and dried at 105 °C for 12 h to measure its oven-dried weight. The separated hot-compressed water-soluble portion was kept standing for 12 h under ambient condition. During this period, it was separated to be water-soluble portion and precipitate which were retrieved by filtration with Millipore (pore size: 0.45 µm). For the water-soluble portion, the lignin-derived compounds were obtained by extraction with ethyl acetate.

Analytical methods

The content of lignin-derived products in each fraction was examined as the combined yields of Klason lignin and acid-soluble lignin [25]. For the acid-soluble portion obtained, the amounts of various mono-saccharides were determined by high performance anion-exchange chromatography. Its cellulose and hemicellulose contents were then estimated based on the amounts of glucose and other mono-saccharides,

respectively [26]. Molecular weight distribution was evaluated by gel permeation chromatography (GPC) analysis, which was performed with LC-10A (Shimadzu, Kyoto, Japan) under the following conditions: column, Shodex KF-801 + KF-802 + KF-802.5 + KF-803 (Showa Denko, Tokyo, Japan); eluent, tetrahydrofuran; flow-rate, 0.6 mL/min; column temperature, 50 °C; detector, Ultraviolet light at 280 nm. For comparison, polystyrene standards (Molecular weight (MW): 162, 580, 1270, 2960, 5000) were used as standard.

In order to evaluate chemical characteristics of lignin-derived products, alkaline nitrobenzene oxidation was performed and gas chromatographic analysis was undertaken to obtain the total yield of vanillin [27]. However, since water-soluble portion contained some monomeric products like coniferyl alcohol, the vanillin yield from oligomeric products was evaluated from the total yield of vanillin minus vanillin from monomeric products, according to the following equation (1):

$$\text{Yield of vanillin from oligomeric lignin-derived products (mmol/g)} = \frac{\text{Total yield of vanillin (mmol)} - \text{Yield of vanillin from monomeric lignin - derived products (mmol)}}{\text{Lignin - derived products (g)} - \text{Monomeric lignin - derived products (g)}}$$

Since monomeric compounds are mostly composed of coniferyl alcohol, coniferyl aldehyde, vanillin and isoeugenol, other monomeric products were ignored in this calculation.

Monomeric and dimeric compounds of lignin-derived products in water-soluble portion were analyzed by gas chromatography – mass spectrometry (GC-MS), which was performed with Hitachi M7000s and M9000 series. The capillary column was a DB-5MS. The temperature program was 1.0 min at 40 °C, 5.0 °C/min to 300 °C, and 8.0 min at 300 °C. Helium carrier gas was used at a flow-rate of 1.5 mL/min. Injector and detector temperatures were both 230 °C.

High molecular weight compounds of lignin-derived products in water-soluble portion were analyzed by matrix-assisted laser desorption ionization-time of flight / mass spectrometry (MALDI-TOF/MS), which was performed with AXIMA Performance (Shimadzu, Kyoto, Japan). Nitrogen gas was used as the laser source. The analysis was performed in positive-ion, linear mode and 2,5-dihydroxybenzaldehyde was used as matrix.

Results and discussion

Lignin-derived products

The temperature change of hot-compressed water treatment for the 1st stage (230 °C/10

MPa/15 min) and 2nd stage (270 °C/10 MPa/15 min) is shown in Fig. 1. The yield of water-insoluble residue and its chemical composition are also shown. When extractive-free Japanese cedar is treated by hot-compressed water, hemicelluloses and a part of cellulose are decomposed and eluted as hot-compressed water-soluble portion at the 1st stage, and then in the 2nd stage, the rest of cellulose is mostly decomposed and eluted. It seems likely that cellulose decomposed in the 1st stage might have lower crystallinity compared with remaining cellulose decomposed in the 2nd stage. In contrast, lignin is decomposed at both stages recovered as lignin-derived products in all fractions. However, some of lignin has much higher resistance to hot-compressed water as compared with cellulose and hemicelluloses. The hot-compressed water-soluble portion eluted is later partially precipitated under ambient conditions.

The yields of water-soluble portion, precipitate, and water-insoluble residue in both stages are summarized in Table 1. In addition, the yield of the lignin-derived products and the lignin concentration in each fraction are also shown. In the 1st stage, 52.1 (= 47.1 + 5.0) % of Japanese cedar was eluted in hot-compressed water, and 5.0 % was obtained as precipitate. Consequently, 47.1 % was obtained in water-soluble portion and 47.9 % remained as water-insoluble residue. After the 2nd stage treatment of the water-insoluble residue (47.9 %), 35.1 (= 29.8 + 5.3) % was eluted in hot-compressed water, and then 5.3 % and 29.8 % were obtained as precipitate and water-soluble portion,

respectively. As a result, 12.8 % of Japanese cedar remained as water-insoluble residue after two-step treatment. This value is, in fact, much higher than that of Japanese beech only to be 4.4 % [22]. The observed difference in lignin between the two woods would be due to the fact that Japanese cedar has less ether linkages than Japanese beech.

The yields of lignin-derived products in each fraction are shown in extractive-free wood-based wt %. For the 32.0 % lignin content of Japanese cedar, 14.0 (= 10.1 + 3.9) % was eluted in hot-compressed water at the 1st stage, with 10.1 % and 3.9 % being from water-soluble portion and precipitate, respectively. In the 2nd stage, 6.8 % (=5.0 + 1.8) was resulted with 5.0 % and 1.8 % as water-soluble portion and precipitate, respectively. On the other hand, 11.2 %, about one third of the initial lignin content, still remained as water-insoluble residue after two-step treatment.

The lignin concentration was 0.214 g/g and 0.168 g/g for water-soluble portion in both 1st and 2nd stages, respectively. For precipitate, the lignin concentration in the 1st stage was 0.780 g/g, whereas that in the 2nd stage was much lower to be 0.340 g/g. This difference could indicate that high molecular weight cellulose-derived products, eluted in the 2nd stage, aggregated and precipitated with lignin-derived products under ambient condition [28]. A similar tendency was observed in the case of Japanese beech [22]. The lignin concentration of the water-insoluble residue increased as the hot-compressed water treatment was prolonged, and the final residue consisted mostly of lignin (0.875

g/g), indicating that the residual lignin has much higher resistance to hot-compressed water than other chemical components.

Structural analysis of lignin-derived products

Fig. 2 demonstrates GPC chromatograms of water-soluble portion and precipitate of both stages, detected at 280 nm in wavelength. In order to evaluate the molecular weight distribution of their lignin-derived products, the elution time of polystyrene standards (MW: 162, 580, 1270, 2960, 5000) were displayed. The higher molecular weight standards show the shorter elution time in GPC chromatograms. The chromatogram of water-soluble portion in the 2nd stage has an intensive peak at around 63 min, which indicates 5-hydroxymethylfurfural derived from polysaccharides.

For the water-soluble portion, the lignin-derived products in the 1st stage contain oligomeric products up to MW of 1270, equivalent to heptamer of coniferyl alcohol, whereas the lignin-derived products in the 2nd stage contain smaller than those in the 1st stage. In contrast, the molecular weight of the precipitate lignin is higher than the lignin-derived products in water-soluble portion for both stages.

In order to compare the relative proportion of ether linkages of lignin-derived products, alkaline nitrobenzene oxidation was conducted, and the obtained yield of its

products from the lignin-derived products is shown in Fig.3. Since *p*-hydroxybenzaldehyde derived from *p*-hydroxyphenylpropane (H) lignin was a trace amount, the yield of vanillin derived from guaiacyl (G) lignin was only shown. For comparison, the yield of vanillin from the untreated Japanese cedar is also shown. Since water-soluble portion contains some monomeric products like coniferyl alcohol as already described, the vanillin yield from oligomeric products was obtained by subtracting vanillin yield from monomeric products from the total yield of vanillin.

For the water-soluble portion, the yields of vanillin from the 1st and 2nd stages are 0.64 and 0.38 mmol/g of original lignin, respectively, both of which are smaller than the vanillin derived from untreated wood (1.53 mmol/g). This suggests that the lignin-derived products of water-soluble portion contain a lower ratio of ether type lignin than untreated wood lignin. On the other hand, the yields of vanillin from precipitate lignin in both stages were comparable to that from untreated wood. This could indicate that precipitate lignin maintains ether linkages. For water-insoluble residue, the yield of vanillin from the 1st stage is 0.63 mmol/g, whereas that from 2nd stage 0.17 mmol/g, decreasing in its yield as the hot-compressed water treatment progressed. Consequently, lignin in the water-insoluble residue, especially for 2nd stage, is rich in condensed type lignin.

Lignin-derived products in water-soluble portion

In order to identify lignin-derived low molecular weight compounds in water-soluble portion, GC-MS analysis was performed. GC-MS spectrum of lignin-derived products in water-soluble portion is shown in Fig. 4. The peaks in the short retention time before 35 min are lignin-derived monomeric compounds, while those in the longer retention time after 35 min are dimeric compounds.

According to the comparison with lignin monomeric model compounds, 17 kinds of lignin-derived compounds such as vanillin (No. 5), isoeugenol (No. 6), coniferyl aldehyde (No. 17), and coniferyl alcohol (No. 18) are identified as shown in Table 2. Some lignin-derived products were identified from their mass spectra by comparing their retention times with those of authentic compounds as in the literature [6,29,30]. Consequently, these identified compounds contained guaiacol skeletons, indicating that the methoxyl group has not been removed from aromatic ring by hot-compressed water treatment. These compounds also contain phenolic hydroxyl group. Most of this hydroxyl group could be produced due to the cleavage of ether linkages by hot-compressed water treatment. Among these identified compounds, *trans*-coniferyl alcohol is obtained as the main product. It would mean that the lignin-derived products in water-soluble portion largely maintain the side-chain structure of lignin. A few of

ketone compounds, which are characteristic products from acidolysis of lignin, are observed in water-soluble portion. Thus, acidolysis reaction can be ignored in the hot-compressed water treatment.

Takada et al. obtained the lignin-derived compounds from Japanese cedar (*Cryptomeria japonica*) by supercritical water treatment (380 °C/100 MPa/8 sec) as methanol-soluble portion, and analyzed the lignin-derived monomeric and dimeric compounds with GC-MS [6]. The lignin-derived monomeric products in methanol-soluble portion contained guaiacol skeletons, indicating that the methoxyl group had not been removed from aromatic ring by supercritical water treatment. In addition, vanillin was mainly obtained without recovered coniferyl alcohol. Thus, the side-chain structure in lignin was more altered by supercritical water treatment compared with hot-compressed water treatment. This suggests that hot-compressed water treatment used in this study can suppress the decomposition of side-chain in lignin more effectively than supercritical water treatment.

Table 3 demonstrates mass fragments and retention time of lignin-derived dimeric compounds. Among these compounds, Nos. 20, 26, and 31 in Fig. 5 are identified from their mass spectra by comparing their retention times with those of authentic compounds as in the literature and the number of hydroxyl groups, estimated from the increase of molecular weight after trimethylsilyl derivatization [6]. These identified

compounds are condensed type, containing methoxyl and phenolic hydroxyl groups as observed in the monomeric compounds in Table 2, whereas Nos. 20 and 26 in Fig. 5 did not maintain the structure of phenylpropane side-chain. Generally, in hydrothermal treatment, formaldehyde produced from lignocellulose forms diarylmethane structure [31]. However, diarylmethane compound could not be found in GC-MS analysis. This could be because semi-flow type treatment minimizes possible formation due to prompt removal of the decomposed products.

In order to identify lignin-derived oligomeric compounds, MALDI-TOF/MS analysis was performed. The obtained MALDI-TOF/MS spectra of the lignin-derived products of the 1st and 2nd stages are shown in Fig. 6. The spectra of the 1st stage contain several molecular weight intervals (MWIs) of 178 and 196. According to the experiment with lignin model compounds, β - β or β -5 linkage results in the MWI of 178 due to the radical mechanism of coniferyl alcohol (MW: 180.20), whereas β -O-4 linkage results in the MWI of 196 due to the addition of hydroxyl group [32]. Besides, some other linkages such as 5-5', 4-O-5 and α -O-4 linkages can result in the MWI of 178 as well. Namely, the MWIs of 178 and 196 obtained from MALDI-TOF/MS spectra of the lignin-derived products in the 1st stage should be derived from coniferyl alcohol, suggesting that the oligomeric lignin-derived products up to heptamers contain both ether and condensed type linkages. In contrast, the spectrum of 2nd stage does not

contain the MWIs of 178 or 196, indicating that the phenylpropane side-chain could be altered by hot-compressed water. The oligomeric lignin-derived products consisted up to tetramer. These results really agree with those from GPC analysis.

Lignin-derived products in precipitate

The lignin-derived products in precipitate in both stages are composed of the higher molecular weight products than water-soluble portion, and their structure was rich in ether linkage according to the yield of vanillin by alkaline nitrobenzene oxidation (Fig. 3). These results suggest that some micropores were produced in the cell wall due to the decomposition of hemicelluloses and the cleavage of ether linkages in lignin in the 1st stage, and the micropores would have facilitated the elution of the lignin cluster, which maintains ether linkages [22]. Hydrophobic lignin could be eluted in hot-compressed water due to the low dielectric constant of hot-compressed water, whereas some large molecules cluster precipitated after hot-compressed water returned to the ordinary water under ambient condition. In the 2nd stage, cellulose- and lignin-derived oligomeric compounds were also eluted in hot-compressed water.

320 Lignin-derived products in water-insoluble residue
321
322 Approximately one third of original lignin still remained as water-insoluble residue after
323 two-step treatment, and the water-insoluble residue is 0.875 g/g in lignin concentration
324 as in Table 1. The ratio of condensed type linkages was observed to increase as the
325 hot-compressed water treatment was prolonged. Consequently, lignin in the
326 water-insoluble residue after two-step treatment was rich in condensed type due to the
327 cleavage of ether type linkages and possible recondensation reaction.

Concluding remarks

Decomposition behavior of lignin in Japanese cedar as treated by two-step semi-flow hot-compressed water was discussed through the analyses of the lignin-derived products fractionated during the treatment.

For the 1st stage (230 °C/10 MPa/15 min), in which hemicelluloses and a part of cellulose were decomposed, approximately half of lignin was decomposed and eluted in hot-compressed water. Due to the decomposition of hemicelluloses and the cleavage of ether linkages in lignin, some micropores would be occurred in the cell wall, which would have facilitated the elution of the lignin cluster together with hemicelluloses-derived products. The hydrophobic lignin could be, thus, eluted in hot-compressed water due to the low dielectric constant of hot-compressed water. After its elution, cluster of some large molecules were precipitated under ambient condition. Since the precipitated lignin contained ether linkages, it would be considered to maintain the structure of original lignin. On the other hand, lignin-derived products in water-soluble portion were low molecular weight up to heptamers, and identified monomeric compounds maintained methoxyl and phenolic hydroxyl groups such as coniferyl alcohol, coniferyl aldehyde and vanillin.

For the 2nd stage (270 °C/10 MPa/15 min), in which the rest of cellulose was mostly

decomposed, a part of lignin was decomposed and eluted. As in the 1st stage, some large molecules of lignin clusters would be eluted and then precipitated under ambient condition.

The rest of the remaining lignin to be one third of the original one had resistance to hot-compressed water and remained as water-insoluble residue, which was mostly composed of lignin. It is interesting that the remaining lignin rich in condensed type as compared with untreated wood can be expected to be utilized as materials resistant to any external attacks.

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Fig. 1 The yield of water-soluble portion, precipitate and water-insoluble residue composed of hemicelluloses, cellulose and lignin as treated by two-step semi-flow hot-compressed water. The treatment temperature change is also shown.

Fig. 2 GPC chromatograms of the water-soluble portion and the precipitate in the 1st stage (upper) and those in the 2nd stage (lower)

Fig. 3 The yield of alkaline nitrobenzene oxidation products for the water-soluble portion, precipitate and water-insoluble residue in the 1st stage and 2nd stage. Just comparison, the yield of the untreated Japanese cedar is included.

Fig. 4 Total ion chromatogram of the water-soluble portion from Japanese cedar as treated by semi-flow hot-compressed water by GC-MS. The numbers correspond to those in Tables 2 and 3.

Fig. 5 Speculative structures of some lignin-derived dimeric products.

Fig. 6 MALDI-TOF/MS spectra of the lignin-derived products in the water-soluble portion for the 1st (upper) and 2nd stages (lower) (m/z : 500-1400). Several molecular weight intervals (MWI) of about 178.20 and 196.20 were obtained from the 1st stage water-soluble portion, suggesting β - β or β -5, and β -O-4 linkages, respectively.

Table 1 The yields of fractionated product and lignin-derived product, and lignin concentration in water-soluble portion, precipitate and water-insoluble residue in the 1st and 2nd stages, as Japanese cedar treated by two-step semi-flow hot-compressed water.

Table 2 Lignin-derived monomeric products in water-soluble portion and their retention times, structures, and MS fragments for GC-MS analysis.

Table 3 Lignin-derived products in water-soluble portion and their retention times and MS fragments for GC-MS analysis.

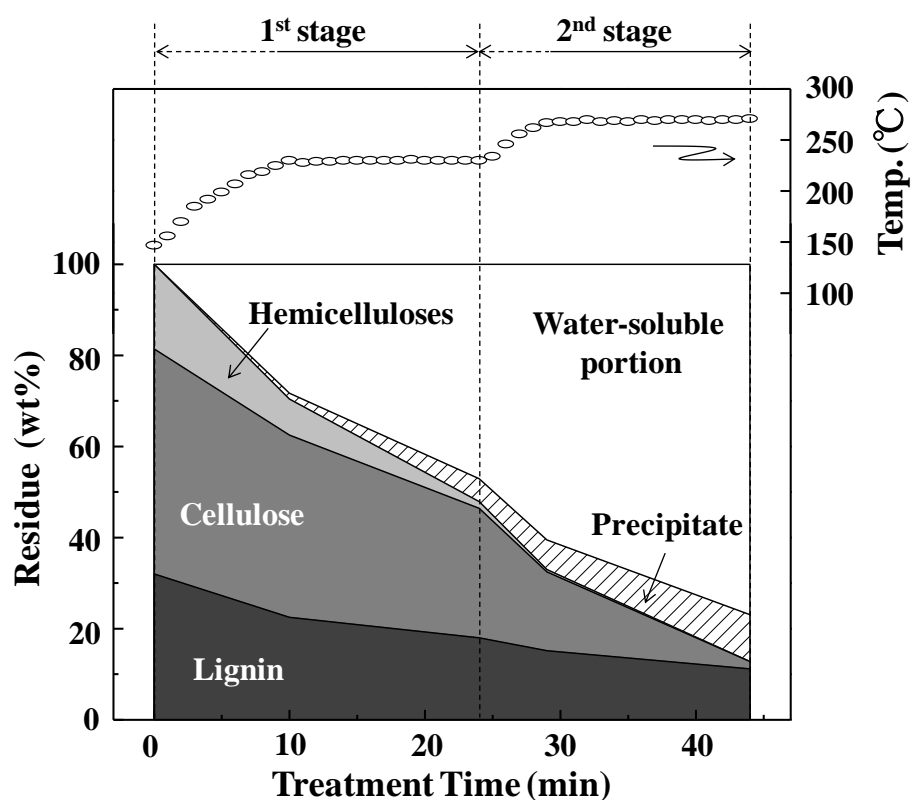


Fig. 1. The yield of water-soluble portion, precipitate and water-insoluble residue composed of hemicelluloses, cellulose and lignin as treated by two-step semi-flow hot-compressed water. The treatment temperature change is also shown.

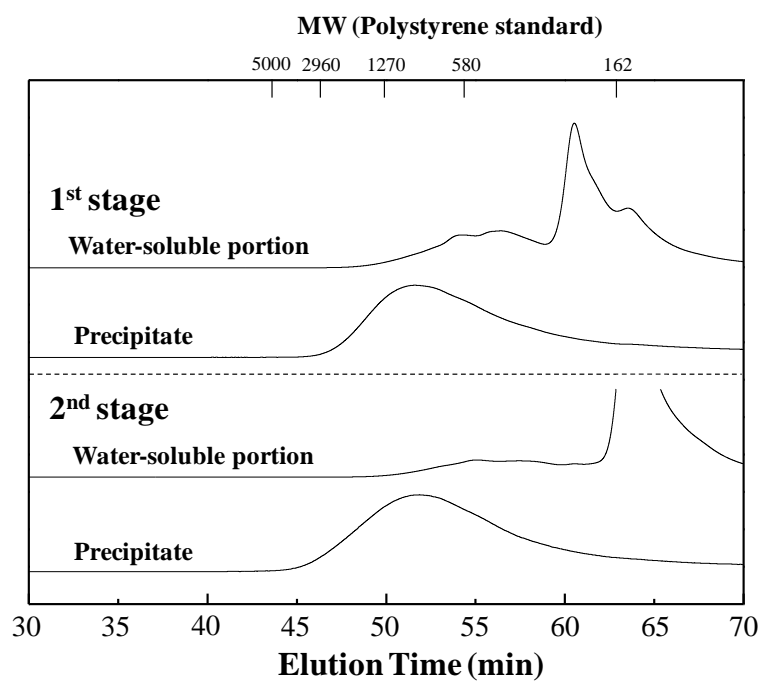


Fig. 2. GPC chromatograms of the water-soluble portion and the precipitate in the 1st stage (upper), and those in the 2nd stage (lower).

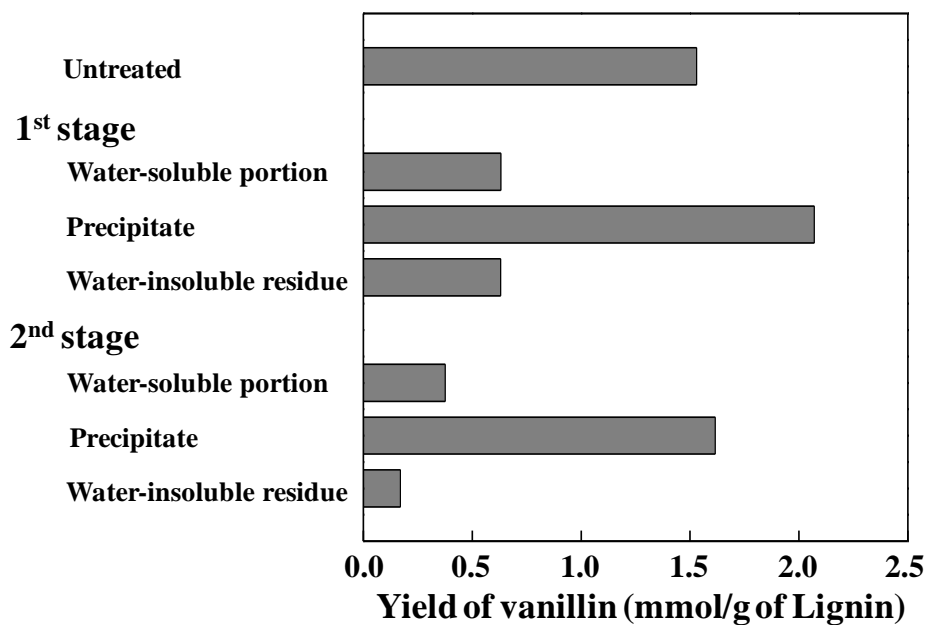


Fig. 3. The yield of alkaline nitrobenzene oxidation products for the water-soluble portion, precipitate and water-insoluble residue in the 1st stage and 2nd stage. Just comparison, the yield of the untreated Japanese cedar is included.

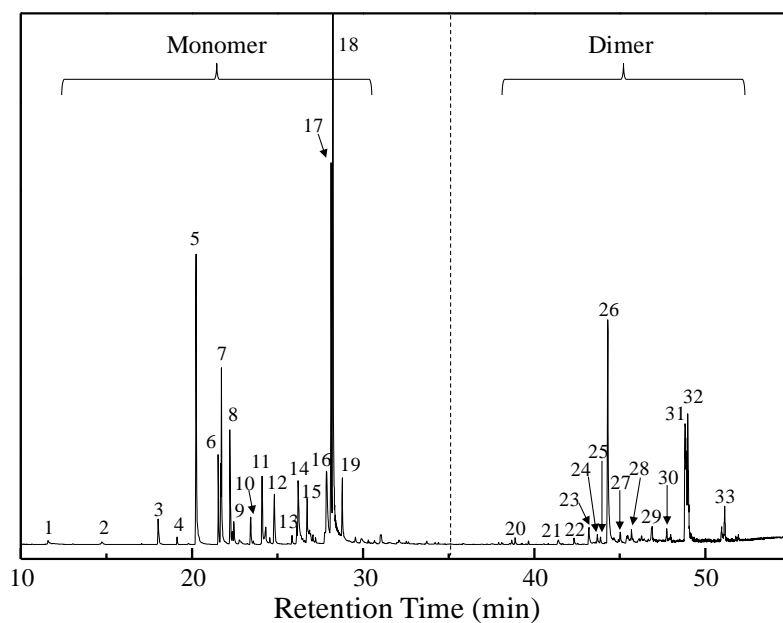
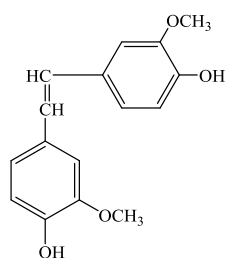


Fig. 4. Total ion chromatogram of the water-soluble portion from Japanese cedar as treated by semi-flow hot-compressed water by GC-MS. The numbers correspond to those in Tables 2 and 3.

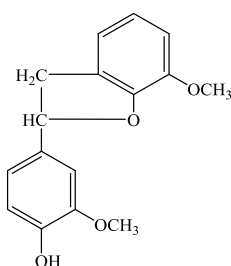
502

No. 20



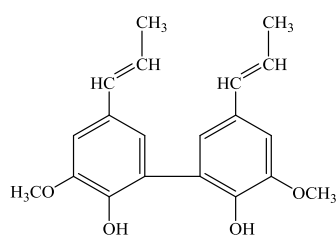
Stilbene type
(β-1)

No. 26



Phenylcoumaran type
(β-5)

No. 31



Biphenyl type
(5-5')

503

504

505 Fig. 5. Speculative structures of some lignin-derived dimeric products.

506

507

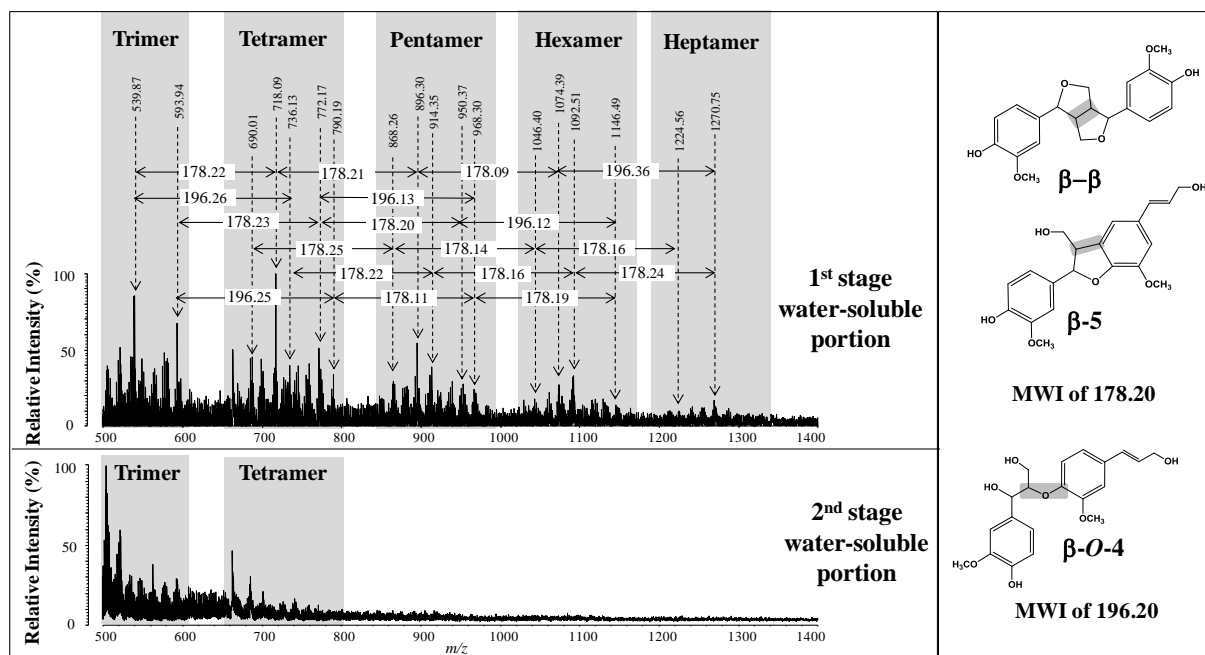


Fig. 6. MALDI-TOF/MS spectra of the lignin-derived products in the water-soluble portion for the 1st (upper) and 2nd stages (lower) (m/z : 500-1400). Several molecular weight intervals (MWI) of about 178.20 and 196.20 were obtained from the 1st stage water-soluble portion, suggesting β - β or β -5, and β -O-4 linkages, respectively.

Table 1. The yields of fractionated product and lignin-derived product, and lignin concentration in water-soluble portion, precipitate and water-insoluble residue in the 1st and 2nd stages, as Japanese cedar treated by two-step semi-flow hot-compressed water.

Stage	Fraction	Yield of fractionated product (wood-based wt%)	Yield of lignin-derived product (wood-based wt%)	Lignin concentration* (g/g)
1 st stage	Water-soluble portion	47.1	10.1	0.214
	Precipitate	5.0	3.9	0.780
	Water-insoluble residue	47.9	18.0	0.376
2 nd stage	Water-soluble portion	29.8	5.0	0.168
	Precipitate	5.3	1.8	0.340
	Water-insoluble residue	12.8	11.2	0.875
Total		100	32.0	-

* Lignin concentration was obtained by the yield of lignin-derived product divided by that of fractionated product.

Table 2. Lignin-derived monomeric products in water-soluble portion and their retention times, structures, and MS fragments for GC-MS analysis.

No.	Product	Retention Time (min)	Structure ^{*a}	MS fragments (<i>m/z</i>)
1	Guaiacol	11.6	G	124, 109, 89, 81
2	Methylguaiacol	14.9	G—C	138, 123, 95, 77
3	Vinylguaiacol ^{*b}	18.0	G—C=C	150, 136, 107, 78
4	Eugenol	19.1	G—C—C=C	164, 149, 134, 104, 77
5	Vanillin	20.3	G—CHO	152, 123, 109, 77
6	<i>trans</i> -Isoeugenol	21.5	G—C=C—C	164, 150, 104
7	Homovanillin	21.7	G—C—CHO	166, 138, 123
8	Unknown	22.2		162, 148, 120, 92
9	Acetovanillone	22.4	G—CO—C	166, 152
10	Guaiacylacetone ^{*c}	23.4	G—C—CO—C	180, 137
11	Unknown	24.1		178, 150, 90, 78
12	2-Methoxy-4-(prop-1-en-3-one)phenol ^{*d}	24.8	G—CO—C—C	178, 151
13	Homovanillic acid	26.1	G—C—COOH	182, 152
14	3-4-Hydroxy-3-methoxyphenyl-1-propanol	26.2	G—C—C—C—OH	182, 138
15	<i>cis</i> -Coniferyl alcohol	26.7	G—C=C—C—OH	180, 163, 138, 124, 78
16	Hydroferulic acid	27.8	G—C—C—COOH	196, 137
17	<i>trans</i> -Coniferyl aldehyde	28.2	G—C=C—CHO	178, 164, 149
18	<i>trans</i> -Coniferyl alcohol	28.4	G—C=C—C—OH	180, 164, 138
19	Ferulic acid	28.8	G—C=C—COOH	194, 167, 138

^{*a}: G: guaiacyl (2-methoxyphenyl)

^{*b}: Product identified according to literature 6

^{*c}: Product identified according to literature 29

^{*d}: Product identified according to literature 30

Table 3. Lignin-derived dimeric products in water-soluble portion and their retention times and MS fragments for GC-MS analysis.

No.	Retention Time (min)	Product	MS fragments (<i>m/z</i>)
20	38.7	Stilbene type (β -1)	272, 240, 226, 212, 198, 180, 170, 152, 138, 77, 66
21	41.4		281, 274, 260, 230, 214
22	42.3		278, 275, 245, 160, 153, 124, 109, 78, 66, 52
23	43.2		290, 152, 139, 124, 108, 94, 78, 66
24	43.7		429, 377, 356, 301, 273, 160, 153, 124, 96, 78, 66, 53
25	43.9	Phenylcoumaran type (β -5)	311, 300, 287, 272, 256, 240, 161, 152, 95, 78, 67
26	44.3		272, 241, 225, 213, 212 199, 169
27	45.0		303, 273, 153, 124, 108, 82, 78, 66, 53
28	45.7		358, 313, 298, 282, 253, 210, 181, 162, 79, 52
29	46.9		357, 341, 313, 286, 269, 208, 175, 160, 152, 137, 78
30	47.7	Biphenyl type (5-5')	344, 326, 312, 207, 139, 121, 93, 78, 66
31	48.8		326, 312, 298, 266, 190, 177, 162, 139, 124, 104, 92, 78
32	49.0		314, 298, 252, 239, 178, 138, 95, 78
33	51.1		327, 324, 312, 135, 130, 78